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Abnormal Proliferation of CD4$^-$ CD8$^+$ γδ$^+$ T Cells With Chromosome 6 Anomaly: Role of Fas Ligand Expression in Spontaneous Regression of the Cells

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We report a case of granular lymphocyte proliferative disorder accompanied with hemolytic anemia and neutropenia. Phenotypes of the cells were T cell receptor γδ$^+$ CD3$^+$ CD4$^-$ CD8$^+$ CD16$^+$ CD56$^-$ CD57$^-$. Southern blot analysis of T cell receptor β and γ chains demonstrated rearranged bands in both. Chromosomal analysis after IL-2 stimulation showed deletion of chromosome 6. Sorted γδ$^+$ T cells showed an increase in Fas ligand expression compared with the levels in sorted αβ$^+$ T cells. The expression of Fas ligand on these γδ$^+$ T cells increased after IL-2 stimulation. The patient's anemia improved along with a decrease in granular lymphocyte count and disappearance of the abnormal karyotype without treatment. The expression of Fas ligand may be involved in spontaneous regression of granular lymphocyte proliferation with hemolytic anemia. Am. J. Hematol. 60:305–308, 1999.

Key words: granular lymphocyte proliferative disorder; hemolytic anemia; Fas ligand; spontaneous regression

INTRODUCTION

Granular lymphocyte proliferative disorder (GLPD) is a distinct clinical entity [1,2]. GLPD has been divided into two subgroups: CD3$^+$ T cell type and CD3$^-$ natural killer cell type. T-granular lymphocytes usually express T cell receptor (TCR) αβ heterodimers, but rarely the alternative TCR γδ (γδ$^+$ T cell). Clonal expansion of γδ$^+$ T cells, being CD4$^-$ CD8$^+$ [3–8] or CD4$^-$ CD8$^-$ [9–11] without involvement of autoimmune diseases, has been reported, but the neoplasmic nature of these cells has not yet been determined.

Apoptosis plays a critical role in the regulation of the immune response [12], and the Fas/Fas ligand (FasL) system has emerged as an important cellular pathway regulating the induction of apoptosis [13]. FasL is expressed predominantly in activated T cells and natural killer cells [14]. Fas has been implicated as being involved in activation-induced suicide of T cells based on the observation of mice lacking a functional Fas system [13].

Herein we report a case with clonal expansion of CD4$^-$ CD8$^+$ γδ$^+$ T granular lymphocytes expressing functional FasL, which improved spontaneously during the clinical course.

CASE REPORT

S.K., a 47-year-old man, was referred to our hospital because of anemia and neutropenia in June 1996. Physical examination revealed no lymphadenopathy. Hemoglobin (Hb) was 9.8 g/dL; reticulocyte count, 105×10^9/L; WBC count, 8.1×10^9/L; neutrophil count, 0.8×10^9/L; lymphocyte count, 7.2×10^9/L; and platelet count, 291×10^9/L. Increased lymphocytes were large and contained azurophilic granules in 58% of the cells. Surface marker analysis revealed that the granular lymphocytes were predominantly TCRγδ$^+$ CD3$^+$ CD4$^-$ CD5$^-$ CD7$^+$ CD8$^+$ CD11b$^-$ CD11c$^+$ CD16$^+$ CD25$^-$ CD38$^+$ CD56$^-$

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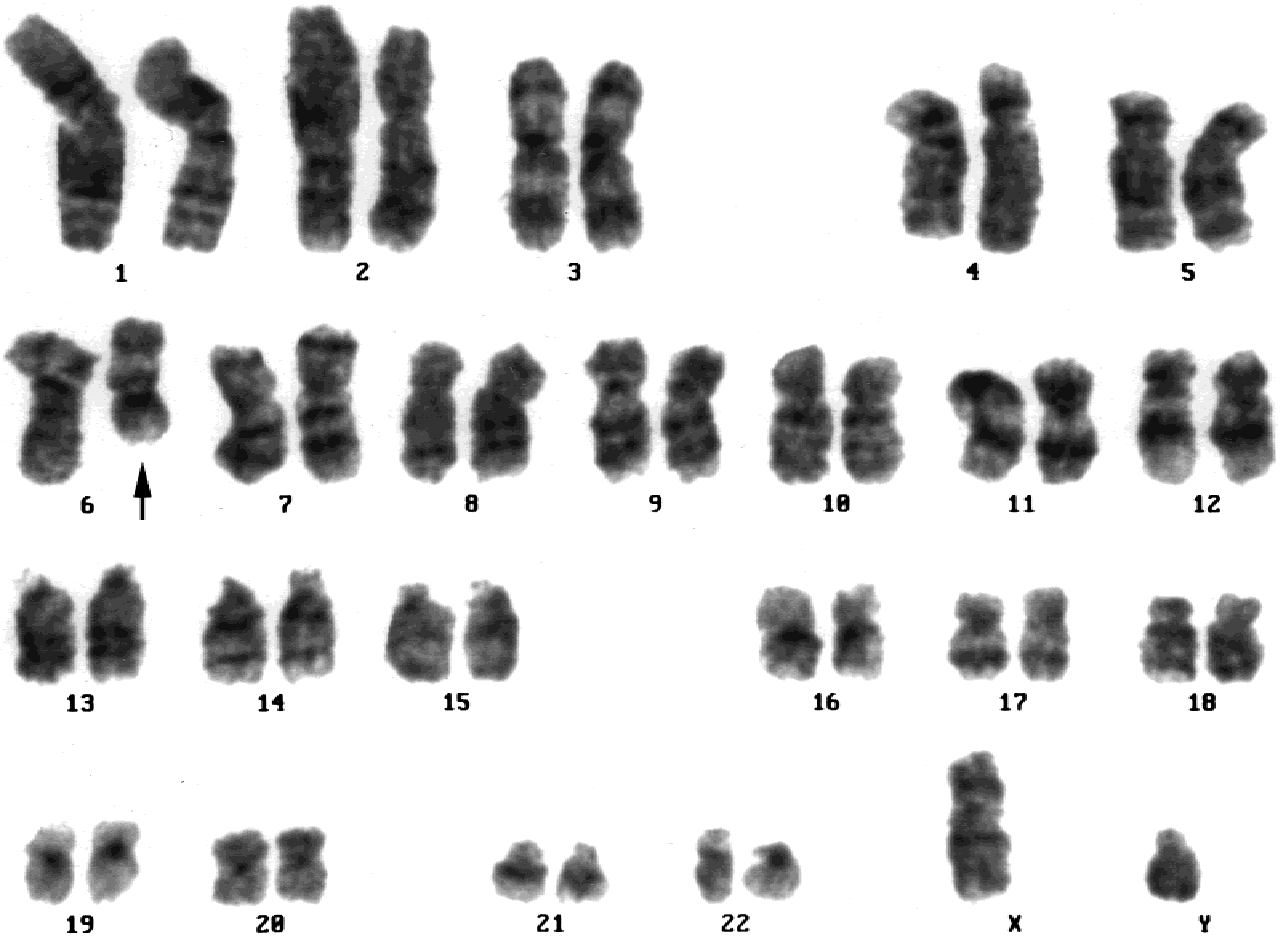

Fig. 1. G-band karyotype of PBMC on IL-2 stimulation, showing 46,XY, del(6)(q?) in 8 (40%) of 20 metaphases.

$CD57^-$ $CD122^-$ $HLA\text{-}DR^+$. Abdominal ultrasonography revealed an enlarged spleen. Chemical data were as follows: TP, 6.4 g/dL; Alb, 4.2 g/dL; T.Bil, 3.6 mg/dL (ID 2.6); AST, 40 U/L; ALT, 36 U/L; LDH, 377 U/L (normal range, 114–220). Haptoglobin was not detectable. Rheumatoid factor, direct Coombs' test, and Ham's test were negative. ^{51}Cr-labeled red blood cell study revealed that erythrocyte life span was shortened (T1/2 = 21.8 days; normal range, 32 ± 2 days). Ferrokinetics studies showed a reduced plasma iron disappearance rate. The patient was diagnosed as having hemolytic anemia and $\gamma\delta^+$ T-GLPD. Chromosomal analysis using PBMC stimulated with 1,000 U/ml interleukin-2 (IL-2) revealed an abnormal karyotype [46,XY, del(6) (q?) (8/20)] (Fig. 1). However, chromosomal abnormalities were not detected by PHA-stimulation. Southern blot analysis of the genes of T cell receptor β and γ chains showed rearranged bands, confirming the clonal proliferation of the T cells. Bone marrow aspirate was normocellular, with a moderate excess of lymphocytes. The count of $CD8^+$ $\gamma\delta^+$ T cells in the peripheral blood gradually decreased accompanied with an improvement of anemia without any need for treatment. The count of $CD8^+$ $\alpha\beta^+$ T cells and $CD56^+$ NK cells in the peripheral blood also gradually decreased, and the count of neutrophil and $CD4^+$ $\alpha\beta^+$ T cells reciprocally increased. Chromosomal analysis after IL-2 stimulation was done repeatedly. The deletion of chromosome 6 was detected in 6/20 metaphases 4 months later, but not detected 8 or 14 months later (Fig. 2). Expression of CD25 and CD122 did not change during the follow-up period.

To purify the $\gamma\delta^+$ T cells, we performed three-color analysis after preincubation with anti-CD56 (Becton Dickinson, Mountain View, CA), anti-αβTCR (Becton Dickinson), and anti-γδTCR (Coulter Immunology, Miami, FL) and sorting of single positive cells with a FACStar plus (Becton Dickinson). We determined the cytotoxic activity of the sorted $\gamma\delta^+$ T cells, $\alpha\beta^+$ T cells, and natural killer (NK) cells at the resting state using a standard 4-hr ^{51}Cr-release assay as described previously [15]. Freshly isolated $\gamma\delta^+$ T cells had negligible spontaneous NK activity against NK-sensitive K562, as in the case of $\alpha\beta^+$ T cells. An increase in the cytotoxic activity against K562, but not in that against NK-resistant Daudi,

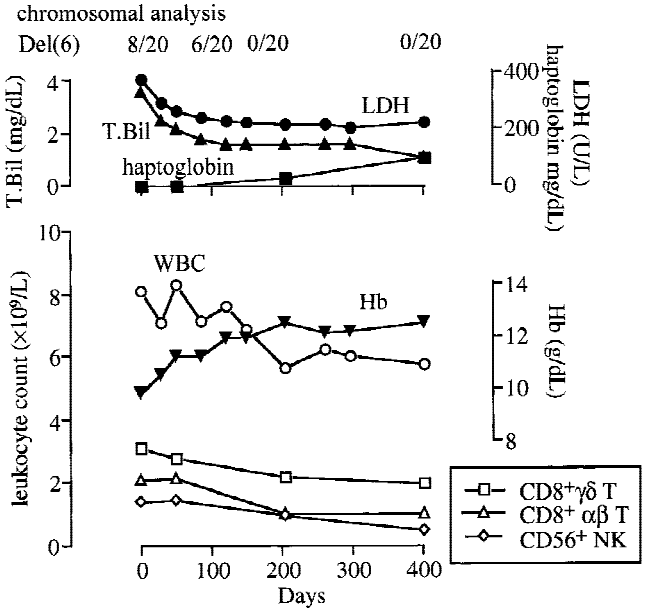

Fig. 2. Clinical course. Changes in the number of Del(6) anomalies detected by chromosomal analysis of PBMC on IL-2 stimulation are shown simultaneously.

could be seen after preincubation of $\gamma\delta^+$ T cells with IL-2 (200 U/mL). IL-2-stimulated $\gamma\delta^+$ T cells also had cytotoxic activity against Fas transfected WR19L (a generous gift from Dr K. Okumura), but none against parental WR19L (data not shown). Next, we examined the FasL expression in sorted $\gamma\delta^+$ T cells and IL-2-stimulated $\gamma\delta^+$ T cells by means of the reverse transcription-polymerase chain reaction (RT-PCR), as described previously [15]. As shown in Figure 3, an increase in FasL expression in sorted $\gamma\delta^+$ T cells compared with that of sorted $\alpha\beta^+$ T cells could be demonstrated. A further increase in FasL expression was noted after IL-2 stimulation.

DISCUSSION

To our knowledge, this is only the fourth reported case of clonal expansion of $CD4^-$, $CD8^+$ $\gamma\delta^+$ T cells. GLPD is often associated with neutropenia and pure red cell aplasia; however, hemolytic anemia, both Coombs' positive and Coombs' negative, is a less frequent manifestation of GLPD [1]. This is the first case of $\gamma\delta^+$ T-GLPD in a patient diagnosed as having Coombs' negative hemolytic anemia. There was no morphological or enzymological abnormality of red cells (data not shown). We quantified red cell-associated IgG (RAIgG) using an immunoradiometric assay as described [16], and revealed no increase in RAIgG, suggesting the hemolytic anemia is not caused by an autoimmune mechanism. Although the mechanism of Coombs' negative hemolytic anemia was unclear in this patient, a correlation between the improvement of anemia and the decrease in GLs suggest that the hemolysis is associated with GLPD. Further analysis is needed to elucidate this issue.

Although the clonal nature of $\gamma\delta^+$ T cells has been reported, cytogenetical abnormalities of these cells have not been demonstrated. We found chromosome 6 anomalies using PBMC stimulated with IL-2, but not with PHA. Morikawa et al. [10] reported that PBMC of a patient with $\gamma\delta^+$ T-GLPD responded well to IL-2, but poorly to PHA, suggesting that metaphase could be obtained easily after IL-2-stimulation in γ/δT-GLPD. Thus, chromosome 6 anomalies may be associated with abnormal $\gamma\delta^+$ T cells. Interestingly, chromosome 6 anomalies disappeared with clinical improvement despite the presence of $\gamma\delta^+$ T cells in the peripheral blood. Chromosome 6q rearrangements are known to be correlated with a poor prognosis in follicular lymphoma [17]. We speculated that some $\gamma\delta^+$ T cells exhibit chromosome 6 anomalies at the onset, and that clonal expansion of these abnormal cells with chromosome 6 anomalies mainly contribute to hemolytic anemia.

Constitutive expression of FasL in abnormal $\gamma\delta^+$ T cells was demonstrated in this study. Previous studies reported that FasL expression may be associated with liver dysfunction or neutropenia in patients with GLPD [18,19]. It has been reported that some patients with GLPD demonstrate spontaneous regression [2]. The fact that chromosomal anomalies in the present case disappeared with clinical improvement suggests that this abnormal clone is activated. Because no method exists to separate living $CD8^+$ $\gamma\delta^+$ T cells with chromosome 6 anomalies from those without chromosome 6 anomalies, we failed to confirm the difference in the FasL expression of these cells. Therefore, we stimulated the sorted $CD8^+$ $\gamma\delta^+$ T cells with IL-2, and confirmed an increase in FasL expression. No difference in FasL expression of $CD8^+$ $\gamma\delta^+$ T cells was detected before or after the regression (data not shown), which may be explained by the low number of activated $CD8^+$ $\gamma\delta^+$ T cells with chromosome 6 anomalies. Because activated T cells can undergo apoptosis mediated by the Fas system [4], functional FasL expression on GLs may contribute to "suicide" or "murder" by protective immunity. The latter possibility is supported by recent findings that FasL-producing tumor cells were rejected in an animal model [20]. Neutrophils and $CD8^+$ T cells are involved in this rejection. As the count of $CD8^+$ $\alpha\beta^+$ T cells and $CD56^+$ NK cells in the peripheral blood increased at the onset in the present case, these cells may contribute to eliminate activated $CD8^+$ $\gamma\delta^+$ T cells. Functional FasL expression may explain the spontaneous improvement in some patients with GLPD. Further analysis is required to elucidate the nature of GLPD.

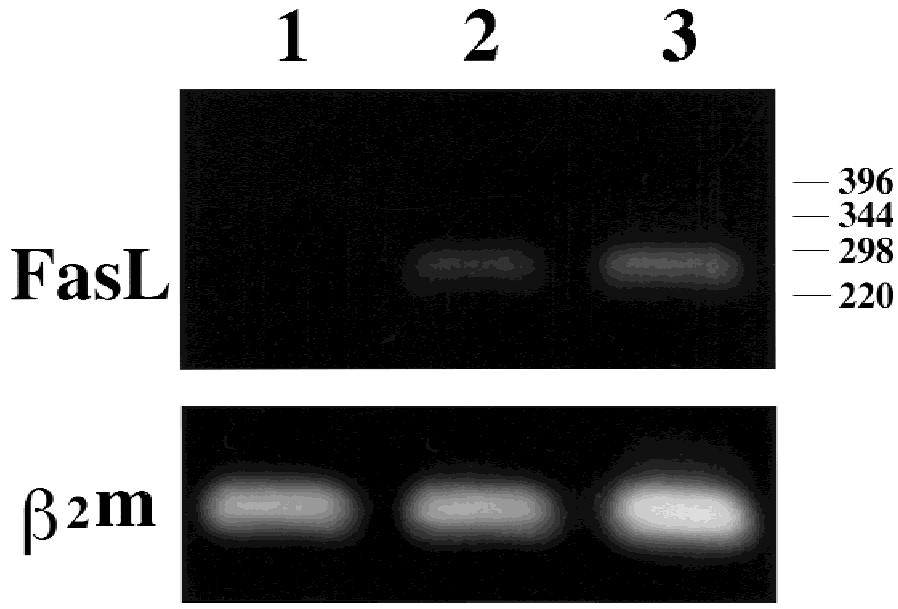

Fig. 3. Expression of FasL mRNA. The expression of FasL and β_2-microglobulin (β_2m) mRNA in freshly isolated $\alpha\beta^+$ T cells (lane 1), $\gamma\delta^+$ T cells (lane 2), 24 hr IL-2 (200 U/mL) activated $\gamma\delta^+$ T cells (lane 3) were analyzed by RT-PCR. Representative data from three independent experiments are shown.

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